

Organochlorine-associated Immunosuppression in Prefledgling Caspian Terns and Herring Gulls from the Great Lakes: An Ecoepidemiological Study

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The objectives of this study were to determine whether contaminant-associated immunosuppression occurs in prefledgling herring gulls and Caspian terns from the Great Lakes and to evaluate immunological biomarkers for monitoring health effects in wild birds. During 1992 to 1994, immunological responses and related variables were measured in prefledgling chicks at colonies distributed across a broad gradient of organochlorine contamination (primarily polychlorinated biphenyls), which was measured in eggs. The phytohemagglutinin skin test was used to assess T-lymphocyte function. In both species, there was a strong exposure-response relationship between organochlorines and suppressed T-cell-mediated immunity. Suppression was most severe (30–45%) in colonies in Lake Ontario (1992) and Saginaw Bay (1992–1994) for both species and in western Lake Erie (1992) for herring gulls. Both species exhibited biologically significant differences among sites in anti-sheep red blood cells antibody titers, but consistent exposure-response relationships with organochlorines were not observed. In Caspian terns and, to a lesser degree, in herring gulls, there was an exposure-response relationship between organochlorines and reduced plasma retinol (vitamin A). In 1992, altered white blood cell numbers were associated with elevated organochlorine concentrations in Caspian terns but not herring gulls. The immunological and hematological biomarkers used in this study revealed contaminant-associated health effects in wild birds. An epidemiological analysis strongly supported the hypothesis that suppression of T-cell-mediated immunity was associated with high perinatal exposure to persistent organochlorine contaminants. — Environ Health Perspect 104(Suppl 4):829–842 (1996)

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Introduction

Laboratory studies have shown that environmental contaminants can suppress immunological function and increase susceptibility to infectious diseases (1–5). Often these chemicals act at low doses and cause persistent effects, especially with perinatal exposure. These laboratory studies raise concerns about potential immunotoxic impacts on wildlife and humans. There have been few immunotoxicological investigations of free-living wildlife, especially birds. We investigated associations between contaminants and immune function in prefledgling fish-eating birds from the Great Lakes.

The high trophic level of fish-eating birds exposes them to elevated concentrations of contaminants that biomagnify. Organochlorines such as polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) have been associated with physiological (6,7), reproductive (8–13), developmental (14–17), behavioral (11,18), and population-level (13,19) problems in fish-eating birds of the Great Lakes during the last 30 years. Although concentrations of many contaminants declined drastically during the 1970s, concentrations have declined slowly, leveled off, or increased more recently (20,21). Reproduction has improved in many areas, but significant biological impacts at highly contaminated sites continue to be associated with coplanar halogenated aromatic hydrocarbons (HAHs) such as TCDD and some PCBs (17).

In laboratory animals, HAHs cause immunosuppression through Ah-receptor-

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Abbreviations used: Ah, aryl hydrocarbon; ANOVA, analysis of variance; C-TEQs, TCDD toxic equivalents determined by the chicken hepatocyte bioassay; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; EDTA, ethylenediaminetetraacetic acid; EROD, ethoxyresorufin-*O*-deethylase; GC-MSD, gas chromatograph-mass spectrometer detector; HAHs, halogenated aromatic hydrocarbons; HCB, hexachlorobenzene; HG-TEQs, TCDD toxic equivalents calculated from herring gull-specific induction equivalency factors; HPLC, high-performance liquid chromatography; IEF, induction equivalency factor; IgG, immunoglobulin G; IgM, immunoglobulin M; ODS, octadecylsilane; PBS, phosphate-buffered saline; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; PHA, phytohemagglutinin; SRBC, sheep red blood cells; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDF, 2,3,7,8-tetrachlorodibenzofuran; WBC, white blood cell.

dependent mechanisms (22–28), although Ah-receptor-independent mechanisms also are involved (27–30). T-lymphocytes, which mature in the thymus, regulate immune responses and attack virus-infected and malignant cells. In chicken embryos, PCB #126 induces the activity of ethoxyresorufin-*O*-deethylase (EROD) in thymic tissue, demonstrating that the avian thymus is a target organ for Ah-receptor-mediated toxicity (31). In developing birds and mammals, low levels of HAHs cause thymic atrophy (22,26,32–35). Toxic effects occur throughout T-lymphocyte development, including the prothymocyte stage in bone marrow (36), thymocyte selection in the thymus (37–40), and the mature T-lymphocyte stage in the blood (41). Numerous T-cell functions are suppressed (32,42–47). B lymphocytes, which mature in the bursa of Fabricius in birds and the bone marrow or Peyer's patches in mammals, produce antibodies that destroy invading microorganisms. High doses of HAHs suppress antibody responses (22,23,28,42,44,45,48–50). HAHs reduce concentrations of retinol [vitamin A] and thyroxine (51–54), which are important for immune function (55–57). Low concentrations of vitamin A occur in herring gulls (*Larus argentatus*) at some highly contaminated Great Lakes colonies (6,58,59).

Immunosuppression by HAHs increases susceptibility to infectious diseases. An early study found increased mortality following challenge with duck hepatitis virus in mallard ducklings (*Anas platyrhynchos*) dosed with PCBs (60). Subsequent laboratory studies showed that HAHs increased susceptibility to bacteria (42,48,61), viruses (26,50,62), and protozoan parasites (26). Several investigators suggested associations between epizootics and elevated organochlorine exposure in beluga whales (*Delphinapterus leucas*) in the St. Lawrence Estuary (63), California sea lions (*Zalophus californianus*) on San Miguel Island (64,65), harbor seals (*Phoca vitulina*) in Europe (66–68), and bottlenose dolphins (*Tursiops truncatus*) in the Atlantic Ocean (69). At highly contaminated sites in the Great Lakes, double-crested cormorants (*Phalacrocorax auritus*) had increased rates of eye infections associated with *Pasteurella multocida* (70). Children exposed perinatally to PCBs and TCDD in arctic Quebec experienced an increased incidence of middle ear infections (71).

For ethical and financial reasons, manipulative field experiments often are

not possible in ecotoxicology. Pollution patterns are determined by the locations of pollution sources and the movement of air, water, and sediments. In the absence of manipulative experiments, epidemiological criteria aid the elucidation of associations between contaminants and biological effects (72). For epidemiologists, establishing causation does not require that a factor be a necessary and sufficient condition to produce an effect. Rather, causal associations imply that a factor is part of a complex that increases the probability of an effect, and that reducing the factor reduces the probability of the effect. Epidemiological criteria for assessing causal associations include time order, strength of association, specificity, consistency upon replication, coherence, predictive performance, and probability (72). The criteria that support an association are weighed against those that detract from it.

The objectives of this ecoepidemiological study were to determine whether contaminant-associated immunosuppression occurs in pre fledgling herring gulls and Caspian terns (*Sterna caspia*) from the Great Lakes and to evaluate immunological biomarkers for monitoring health effects in wild birds. We employed two *in vivo* tests of immune function: the phytohemagglutinin (PHA) skin test for T-cell-mediated immunity and the sheep red blood cell (SRBC)

hemagglutination test for antibody-mediated immunity. We also measured white blood cell (WBC) numbers, plasma retinol, and thyroxine as general biomarkers relevant to immune function. Prefledgling birds were studied because the developing immune system is particularly sensitive to contaminants. The herring gull was chosen because it is the most frequently used and best understood avian bioindicator species in the Great Lakes. The Caspian tern was chosen because its elevated exposure and sensitivity to organochlorines have been associated with reproductive and population-level effects (12,19,73,74). Contaminant-associated immunosuppression in young Caspian terns is a possible mechanism for these population-level effects.

Methods

Sampling Design

In 1992, herring gull chicks were sampled across a gradient of organochlorine contamination at four sites within the Great Lakes and one site outside the Great Lakes (Tables 1,2; Figure 1): *a*) Little Charity Island in Saginaw Bay, Lake Huron; *b*) Bird and Anchor Islands in the North Channel, Lake Huron; *c*) Monroe, on the western shore of Lake Erie; *d*) Hamilton Harbor on the western shore of Lake Ontario; and *e*) Pony Island in northern

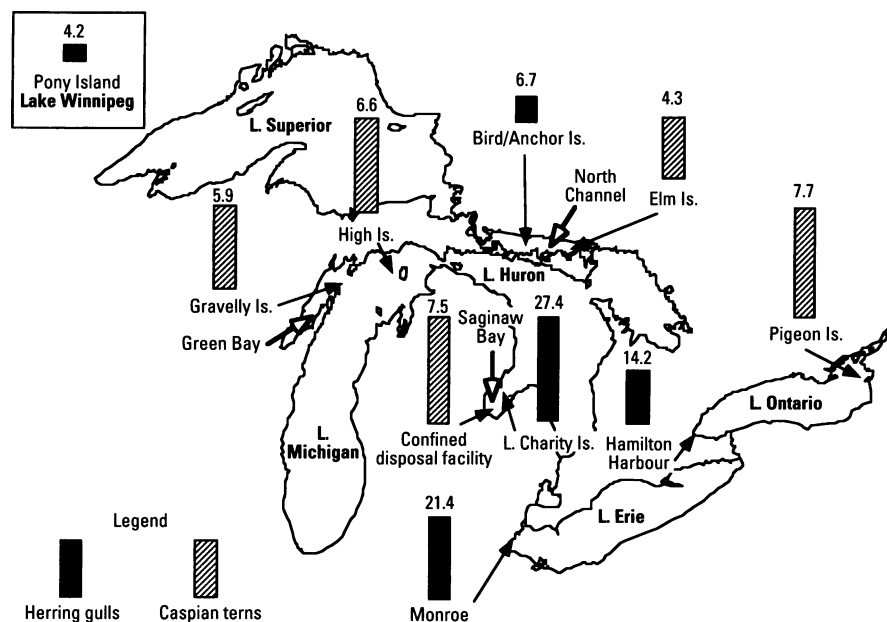


Figure 1. Sampling sites for immune function tests in herring gull and Caspian tern chicks. Bars represent total PCB concentrations found in pooled egg homogenates from each site. Numbers above bars indicate total PCB concentrations. PCB concentrations for the two species are graphed on different scales.

Table 1. Sampling design for immune function study in fish-eating birds of the Great Lakes during 1992 to 1994.

Species/colony and location	Code	Year	Variable				
			PHA test	SRBC test	WBC counts	Retinol	Thyroxine
Herring gull							
Pony Island, northern Lake Winnipeg	Winn	1992	X		X	X	X
Bird Island/Anchor Island, North Channel, Lake Huron	NCh	1992	X		X	X	X
		1993	X	X	X	X	X
Hamilton Harbour, western Lake Ontario	HamH	1992	X	X	X	X	X
Monroe, western Lake Erie	WErie	1992	X	X	X	X	X
Little Charity Island, Saginaw Bay, Lake Huron	SagB	1992	X	X	X	X	X
		1993	X	X	X	X	X
		1994	X	X			
Caspian tern							
Elm Island, North Channel, Lake Huron	NCh	1992	X	X	X	X	X
		1993	X	X	X	X	X
		1994	X	X			
Gravelly Island, Upper Green Bay, Lake Michigan	UGB	1992	X		X	X	X
High Island, northern Lake Michigan	Mich	1992	X	X	X	X	X
Confined Disposal Facility, Saginaw Bay, Lake Huron	SagB	1992	X	X	X	X	X
		1993	X	X	X	X	X
		1994	X	X			
Pigeon Island, eastern Lake Ontario	EOnt	1992	X		X	X	X

Abbreviations: PHA, phytohemagglutinin; SRBC, sheep red blood cells; WBC, white blood cells.

Table 2. Organochlorine contaminants in pooled samples of herring gull and Caspian tern eggs from the Great Lakes and Lake Winnipeg during 1992.

Species/ site code ^a	Concentration (wet weight)								
	ΣPCBs (μg/g)	C-TEQs (ng/g)	HG-TEQs (pg/g)	TCDD (pg/g)	<i>p,p'</i> -DDE (μg/g)	Dieldrin (μg/g)	Mirex (μg/g)	HCB (μg/g)	Heptachlor epoxide (μg/g)
Herring gull									
Winn	4.17	2.60	71	5.3	1.00	0.11	0.03	0.03	0.06
NCh	6.67	3.18	181	16.8	4.03	0.31	0.12	0.04	0.12
HamH	14.18	9.22	240	29.3	5.21	0.07	0.60	0.04	0.04
WErie	21.39	13.48	257	13.1	5.79	0.20	0.08	0.04	0.10
SagB	27.45	17.53	421	35.6	7.78	0.19	0.06	0.05	0.11
Caspian tern									
NCh	4.31	3.29	NA	6.3	0.93	0.09	0.02	0.01	0.03
UGB	5.88	1.62	NA	0.2	2.27	0.16	0.02	0.01	0.06
Mich	6.57	4.38	NA	5.9	3.46	0.16	0.02	0.01	0.06
SagB	7.54	6.84	NA	11.9	3.12	0.02	0.03	0.01	0.02
EOnt	7.73	3.49	NA	12.4	3.78	0.004	0.60	0.01	0.02

Abbreviations: ΣPCBs, total PCBs; C-TEQs, TCDD toxic equivalents determined by the chicken hepatocyte bioassay; HG-TEQs, TCDD toxic equivalents calculated from herring gull-specific induction equivalency factors; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; *p,p'*-DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; HCB, hexachlorobenzene; NA, not applicable. ^aRefer to Table 1 for site codes.

Lake Winnipeg, Manitoba. The North Channel and Lake Winnipeg sites were chosen as reference colonies based on contaminant concentrations previously measured in eggs. Herring gulls were resampled at one reference site, the North Channel, in 1993 and at one highly contaminated site, Saginaw Bay, in 1993 and 1994. In 1992, Caspian tern chicks were sampled at five sites within the Great Lakes (Tables 1, 2; Figure 1): *a*) Gravelly Island in upper Green Bay, Lake Michigan; *b*) High Island in northern Lake Michigan; *c*) Elm Island in the North Channel, Lake Huron; *d*) the Confined Disposal Facility in southern Saginaw Bay, Lake Huron; and *e*) Pigeon Island in eastern Lake Ontario. The North

Channel was chosen as a reference colony. In 1993 and 1994, Caspian terns were resampled at the reference site, the North Channel, and at one highly contaminated site, Saginaw Bay. Logistical difficulties prevented some variables from being measured at some sites (Table 1).

At 8 of 10 sites, enclosures (1 × 2 cm plastic mesh and approximately 0.8 m high) were placed around groups of 10 to 20 herring gull nests or 30 to 40 Caspian tern nests during midincubation. Usually two or three enclosures were erected at each site. Chicks were confined until fledging or until the enclosures were removed. Chicks were banded with U.S. Fish and Wildlife Service leg bands for individual

identification. At two sites, rocky ground prevented the construction of enclosures, so chicks were captured and released into thick vegetation that provided refuge and prevented chicks from fleeing too far from their nests. Immune function tests were initiated on 35 to 50 chicks at each site. WBC numbers, retinol concentrations, and thyroxine concentrations were assessed for 10 to 20 chicks per site.

Immune function tests were initiated on 3-week-old chicks. Age was determined by estimated hatch times and body size measurements. Target body size for herring gull chicks was a body mass of 400 to 700 g and a wing chord of 130 to 200 mm. Criteria for Caspian terns were a

body mass of 450 to 550 g and a wing chord of 130 to 200 mm. Body size measurements were made on the same chicks at the beginning and end of the functional tests.

Functional Tests for Immunocompetence

The PHA skin test for T-cell-mediated immunity was conducted in 3-week-old chicks following the procedures of Grasman and Scanlon (75) using a 0.1-ml dose of 1 mg/ml PHA-P (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS). Feathers were plucked from both wing webs. One wing web was injected with PHA while the other received a placebo injection of PBS alone. The thickness of each wing web was measured to the nearest 0.05 mm immediately before and 24 ± 3 hr after the injections using a pressure-sensitive caliper with a low-tension spring that did not crush the skin (model 304-196, Dyer Co., Lancaster, PA). A stimulation index was calculated as the change in the thickness of the PHA-injected wing web minus the change in thickness of the PBS-injected wing web.

The SRBC hemagglutination test was initiated at the same time as the PHA skin test. Chicks were injected via the wing vein with 0.1 ml of a 1% SRBC suspension in sterile saline. Plasma samples were collected from chicks 5 to 7 days after SRBC injection because antibody titers peak in gulls and terns at approximately 6 days post-immunization (K Grasman, unpublished data). Total (IgM + IgG) and 2-mercaptoethanol-resistant (IgG) antibody activities were measured by the microtiter method of Gross and Siegel (76,77). Fifty microliters of normal saline were added to each well in 96-well microtiter plates with round-bottomed wells. Fifty microliters of plasma were added to the first well of each row, and serial 2-fold dilutions were performed across rows. Fifty microliters of a 0.25% SRBC suspension in normal saline were added to each well. The plates were incubated for 3 hr at 37°C. Titers were determined as the \log_2 of the reciprocal of the highest dilution showing agglutination. To measure IgG titers, plasma samples were incubated for 60 min with 0.2 M 2-mercaptoethanol before dilution. Red blood cells from one sheep (Colorado Serum Co, Denver, CO) were used for all injections and assays during 1992 and 1993. In 1994, SRBCs were obtained from another sheep of the same age and flock.

General Immunological and Hematological Biomarkers

One day after initiating the functional tests, a 4-ml blood sample was drawn from the wing vein of the same chicks using a 22-gauge needle and Vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) (Beckton Dickinson, Rutherford, NJ). Two blood smears were made within 5 hr after blood collection. Blood was centrifuged at 2575×g for 5 min, and the plasma was stored in liquid nitrogen for retinol and thyroxine determinations. A second 4-ml blood sample was collected 6 days after SRBC immunization, and the plasma was stored in liquid nitrogen for antibody analysis.

Blood smears were fixed with methanol and stained with Wright stain (Accustain, Sigma, St. Louis, MO) using 100% stain for 30 sec followed by a 1:1 dilution with distilled water for 90 sec. Smears were rinsed with distilled water and allowed to air dry. Two hundred WBCs were counted and classified using oil immersion microscopy at 1000× magnification.

Retinol was extracted from plasma after the addition of retinyl acetate as an internal standard. Retinol-protein complexes were dissociated by vigorous shaking after the addition of acetonitrile. The retinol was extracted with hexane, and the organic and aqueous phases were separated by centrifugation. The organic phase was dried under nitrogen, reconstituted with methanol, and filtered. The retinoids in the extract were separated by reverse-phase high-performance liquid chromatography (HPLC) using a 15-cm long, 5 μ m octadecylsilane (ODS) analytical column and 100% methanol as a solvent. Either fluorescence (ex: 336 nm; em: 480 nm) or UV-visible (326 nm) was used to detect the retinoids. The detection limit for retinol in plasma was 5 μ g/liter. Total plasma thyroxine was measured using a competitive binding enzyme immunoassay (veterinary modification of the EZ Bead T4 Test, Immunotech Corp, Boston, MA).

Organochlorine Analysis and Chick Hepatocyte Bioassay

The 12 eggs collected from each site were pooled for organochlorine analysis by the analytical services laboratory at the National Wildlife Research Centre of the Canadian Wildlife Service following the methods of Norstrom et al. (78). PCB residues are reported as the sum of the following 42 PCB congeners: IUPAC nos. 28, 31, 42, 44, 49, 52, 60, 64, 66, 70, 74, 87, 97, 99, 101, 105, 110, 118, 128, 129,

137, 138, 141, 146, 149, 151, 153, 158, 170, 171, 172, 174, 180, 182, 183, 185, 194, 195, 200, 201, 203, and 206.

Non-*ortho* PCB congeners (IUPAC nos. 37, 77, 126, and 169) and all 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) also were measured. Samples of the pooled egg homogenates were dried in anhydrous sodium sulfate and ground into a powder. An open chromatographic column wet-packed with multiple absorbents was used for the initial extraction and cleanup. After spiking with a ^{13}C -PCDD mixture (Cambridge Isotope Laboratories) and a ^{13}C -PCB 77, 126, and 169 mixture (Wellington Isotope Laboratories), the column was eluted using dichloromethane/hexane. A carbon column was used for further cleanup and trace enrichment. The concentrated eluent was cleaned up and separated on a deactivated Florisil column by first eluting with dichloromethane/hexane for non-*ortho* PCB analysis. The column was eluted with dichloromethane to produce a second fraction. This fraction was cleaned up on an activated basic alumina column by eluting with 1:50 dichloromethane:hexane to produce a fraction containing residual PCBs and other organochlorines. The column was eluted with dichloromethane:hexane to produce a fraction for PCDD/PCDF analysis. A Hewlett-Packard 5971A GC/MSD was used to separate and quantify non-*ortho* PCB, PCDD, and PCDF congeners. Detection limits were 75 pg/g for non-*ortho* PCB congeners and approximately 0.3 to 2.7 pg/g for PCDD and PCDF congeners.

Because different HAH congeners have different toxicities, the total biological activity of a mixture of congeners cannot be estimated by adding the concentrations of the individual congeners. The chicken embryo hepatocyte bioassay was used to measure the total TCDD-like activity in the pooled egg homogenates (79,80). HAHs were extracted using minor modifications of the procedures used for chemical residue analysis (81,82). Extracts added to chicken hepatocytes contained all PCDDs, PCDFs, PCBs, and structurally related nonpolar HAHs and chlorinated pesticides. Based on the *in vitro* induction of EROD in chicken hepatocytes, this bioassay compared the potency of a mixture of HAHs to that of a TCDD standard. The resulting measure of TCDD-like toxicity was designated as C-TEQs (chicken bioassay-derived TCDD-equivalents).

Kennedy et al. (83) used *in vitro* induction of EROD activity in primary hepatocyte cultures from 26-day-old herring gull embryos to compare the relative toxicities of TCDD, 2,3,7,8-tetrachlorodibenzofuran (TCDF), and various PCBs. Based on the EC₅₀ for EROD induction, different HAH congeners were compared to TCDD, the most toxic congener. The following herring gull-specific induction equivalency factors (IEFs) were generated: TCDD = 1.0; TCDF = 0.9; PCB congener #169 = 0.07, PCB congener #126 = 0.06; PCB congeners #77, #105, #118 = 0. Multiplying the concentration of each congener by its IEF and then summing the products gave an estimate of the total dioxinlike toxicity of the mixture for herring gulls. This estimate was called HG-TEQs (herring gull-specific TCDD-equivalents).

Statistical Analyses

The primary goal of this investigation was to determine whether there was an association between organochlorine exposure and immunosuppression based on intercolony differences in immunological variables. The purpose was not to show what percent of variability in immunological responses could be explained statistically by particular chemicals, but rather to determine the probability that the spatial patterns in response variables were associated with contaminants as opposed to other factors or random events. The strategy for statistical analysis was shaped by two factors: *a*) the importance of testing specific hypotheses regarding associations between immunological variables and organochlorine contaminants, and *b*) the great expense and tissue volume required for congener-specific PCB analysis. The expense and volume requirement prevented contaminant analyses on tissues from individual birds. Instead, sites were ranked in order of contamination for various chemicals based on analysis of the pooled egg homogenates.

The Jonckheere test for ordered alternatives was used to test specific hypotheses concerning contaminant-associated immunosuppression (84). It fit the purpose and design of the study and the constraints on chemical analysis. The null hypothesis for this nonparametric measure of exposure-response states that there is no difference among the central tendencies from different sites ($H_0: \mu_1 = \mu_2 = \mu_3 = \dots = \mu_n$). The alternative hypothesis is that there is a monotonic trend (not necessarily linear) based on a priori information ($H_A: \mu_1 \mu_2 \mu_3 \dots \mu_n$, where at least one of the

inequalities is a strict inequality). We used data from laboratory and other field studies to predict the effects of various contaminants. Inverse relationships to contamination were predicted for the PHA skin response, antibody responses, plasma retinol, and thyroxine. The direction of the trends for WBC numbers could not be predicted from laboratory data, so a two-way test was performed by running the Jonckheere test in both directions and doubling the *p*-value for the most significant trend. Concentrations of total PCBs, DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene), C-TEQs, and HG-TEQs in the egg homogenates were used to determine the relative order of the study sites. The Jonckheere test was performed using a custom-written FORTRAN program (B. Collins, Senior Statistician, Canadian Wildlife Service) using the algorithm provided by Gibbons (85). This program determined probability values based on 5000 random permutations of the data set being analyzed. These probability values were very similar to those found by the large sample normal approximation for the Jonckheere test (84).

Before the Jonckheere test, a preliminary ANOVA was used to determine whether the response variable was influenced by year or a year \times site interaction for subsets containing multiple years of data. If there was no statistically significant year or a year \times site interaction effect ($p < 0.05$), then data were pooled across years for the Jonckheere test. If the Jonckheere test did not show any statistically significant trends, then one-way ANOVA within years followed by Duncan's multiple range test was used to elucidate spatial differences in response variables. For ANOVA analyses, heterophil/lymphocyte ratios were transformed (\log_{10}) to satisfy assumptions of homogenous variances and normality (86). This transformation also was used for the Jonckheere and correlation analyses for this variable. Pearson's correlation analysis was used to detect associations between biomarkers, especially to determine whether any biochemical biomarkers could serve as surrogates for measuring immune function. Statistically significant correlations ($p < 0.05$) were reported only if the absolute value of the correlation coefficient (*r*) was greater than 0.3.

Results

Growth

In Saginaw Bay during 1992, both species experienced severe loss of body mass

between 3 and 4 weeks of age (-11 g/day in herring gull and -5 g/day in Caspian tern chicks), which was much lower than the growth at other sites (14 – 20 g/day for herring gulls, $F_{4,62} = 7.69$, $p < 0.0001$; 4 – 18 g/day for Caspian terns, $F_{3,78} = 16.1$, $p < 0.0001$). Loss of body mass occurred despite abundant food, as assessed by the number of regurgitated food items and fresh pellets. High mortality of chicks and low rates of fledging accompanied this loss of body mass. In Saginaw Bay herring gulls, mean growth recovered significantly to 18 g/day in 1993 and 8 g/day in 1994 ($F_{2,85} = 20.8$, $p < 0.0001$). At Saginaw Bay and in the North Channel, there was a significant year \times site interaction during 1992 to 1994 ($F_{2,180} = 3.23$, $p = 0.042$) with improvement of growth to 4 g/day at Saginaw Bay during 1993 to 1994.

Organochlorine Concentrations

The range in organochlorine concentrations among sites was greater for pooled herring gull eggs than for Caspian tern eggs (Table 2; Figure 1). In herring gulls, total PCBs, C-TEQs, HG-TEQs, and DDE gave the same rank order, so the Jonckheere test was identical for these contaminants. In Caspian terns, the rank orders of contamination were different, requiring separate Jonckheere tests.

Functional Tests For Immunocompetence

In both herring gull and Caspian tern chicks, several measures of organochlorine contamination showed strong inverse exposure-response associations with T-cell function as measured by the PHA skin test (Table 3; Figure 2). For herring gulls, a preliminary ANOVA indicated no evidence for a year or a year \times site interaction effect ($p > 0.45$), allowing data to be pooled across years. There was strong evidence that the PHA response decreased as total PCBs, C-TEQs, HG-TEQs, and DDE increased ($p = 0.0002$ for 1992 to 1994, $p = 0.0009$ for 1992). The most contaminated sites (Saginaw Bay, western Lake Erie, and Hamilton Harbor) were suppressed 35 to 45% compared to the least contaminated sites.

For Caspian terns, a preliminary ANOVA revealed marginal evidence that year affected the PHA response ($F_{2,206} = 2.87$, $p = 0.058$), but this effect was much weaker than the site effect ($F_{1,206} = 31.1$, $p < 0.0001$). During 1992 to 1994, there was strong evidence that the PHA response decreased as total PCB

Table 3. Effects of contaminants on immune function in herring gull and Caspian tern chicks from the Great Lakes and Lake Winnipeg during 1992 to 1994.

Dependent variable	Independent variable	Species	Year	Predicted trend	Actual trend	Tests for ordered alternatives	
						Jonckheere statistic	p-value
PHA skin test	ΣPCBs	Herring gull	1992–1994	–	–	3327	0.0002
	C-TEQs		1992	–	–	603	0.0009
	HG-TEQs	Caspian tern	1992–1994	–	–	10,211	0.0002
	DDE ^a		1992	–	–	1821	0.0004
	ΣPCBs	Caspian tern	1992–1994	–	–	8543	0.0002
	C-TEQs		1992	–	–	387	0.14
	DDE	Caspian tern	1992–1994	–	–	7073	0.0002
			1992	–	–	1589	0.0008
		Herring gull	1992–1994	–	+	–463	0.56
			1992	–	+	–99	0.73
Total antibody response	ΣPCBs	Caspian tern	1992–1994	–	+	–3546	0.99
	C-TEQs		1992	–	+	–602	1.0
	HG-TEQs	Caspian tern	1992–1994	–	+	–5698	1.0
	DDE ^a		1992	–	+	–582	1.0
	ΣPCBs	Caspian tern	1992–1994	–	+	–5698	1.0
	C-TEQs ^a		1992	–	+	–582	1.0

Organochlorine contaminants were measured in pooled egg samples. ^aWhen sites were ranked by different contaminants, their rank orders were identical for several chemicals. Therefore, the Jonckheere test was the same for these chemicals.

($p=0.0002$), C-TEQ ($p=0.0002$), and DDE ($p=0.0002$) exposure increased. In 1992, there was strong evidence for an inverse relationship with total PCBs ($p=0.0004$) and DDE ($p=0.0008$) but not with C-TEQs ($p=0.14$). The most contaminated sites (Saginaw Bay and eastern Lake Ontario) were 30% lower than the least contaminated sites.

In both species, there was no evidence for contaminant-associated suppression of the total antibody (IgM + IgG) and IgG responses following immunization with SRBC ($p>0.50$; Table 3; Figure 3). However, these biomarkers were influenced by site and (or) year. In herring gull chicks of Saginaw Bay, the only site with multiple years of data, there was marginal evidence that total antibody titers decreased from 1992 to 1994 ($F_{2,84}=2.50$, $p=0.088$) but stronger evidence for decreasing IgG titers ($F_{2,84}=4.13$, $p=0.020$). During 1992 to 1994, there was little evidence for a difference among the four sites in total antibody ($F_{3,135}=1.74$, $p=0.16$) or IgG ($F_{3,135}=1.53$, $p=0.21$) titers. In 1992 alone, there was moderate evidence that site influenced total antibody titers ($F_{2,43}=3.97$, $pp=0.026$) and stronger evidence that site influenced IgG titers ($F_{2,43}=7.06$, $p=0.0022$). For Caspian terns from Saginaw Bay and the North Channel, there was strong evidence that year influenced total antibody titers ($F_{2,180}=7.68$, $p=0.0006$) and marginal evidence for a year \times site interaction ($F_{1,180}=2.57$, $p=0.079$). There was strong

evidence for a year \times site interaction effect on IgG ($F_{2,179}=5.24$, $p=0.0061$). In 1992, there was strong evidence for differences among the three sites in total antibody ($F_{2,61}=5.19$, $p=0.0083$) and IgG ($F_{2,61}=5.76$, $p=0.0051$) titers. After examining the differences in titers among Caspian tern colonies, a posteriori Jonckheere tests provided evidence for positive associations between the total antibody response and total PCBs, C-TEQs, and DDE in 1992 alone and 1992 to 1994 ($p<0.02$ for two-way tests).

Biochemical and Hematological Biomarkers

Several organochlorines showed inverse exposure–response relationships with plasma retinol, especially in Caspian tern chicks (Figure 4). In terns from Saginaw Bay and the North Channel, a preliminary ANOVA revealed marginal evidence for a year \times site interaction ($F_{1,31}=3.35$, $p=0.077$) but little evidence for a year effect ($F_{1,31}=2.16$, $p=0.15$). During 1992 to 1993, there was strong evidence for a negative relationship between plasma retinol and total PCBs ($p=0.0002$), C-TEQs ($p=0.0002$), and DDE ($p=0.0002$). In herring gull chicks from Saginaw Bay and the North Channel, a preliminary ANOVA provided little or no evidence that year ($F_{1,36}=1.91$, $p=0.18$) or a year \times site interaction ($F_{1,36}=0.057$, $p=0.81$) influenced plasma retinol. During 1992 to 1993, there was moderate evidence that

plasma retinol decreased as total PCBs, C-TEQs, HG-TEQs, and DDE increased ($p=0.014$).

There was no evidence that organochlorines influenced plasma thyroxine concentrations ($p>0.15$; Figure 5), although these biomarkers were affected by site and (or) year. In herring gull chicks from Saginaw Bay and the North Channel, the preliminary ANOVA indicated strong evidence that a year \times site interaction influenced plasma thyroxine ($F_{1,36}=8.05$, $p=0.007$). In 1992, there was strong evidence that plasma thyroxine differed among the five sites ($F_{4,44}=8.60$, $p<0.0001$). For Caspian tern chicks, there was no evidence for a year \times site interaction effect ($F_{1,33}=0.16$, $p=0.69$) and only marginal evidence for a year effect ($F_{1,33}=3.15$, $p=0.085$) on plasma thyroxine in Saginaw Bay and the North Channel. During 1992 to 1993, there was strong evidence that plasma thyroxine differed among the five sites ($F_{4,64}=7.52$, $p<0.0001$).

Differential WBC counts varied significantly among sites, but an association with organochlorine concentrations was evident only for Caspian tern chicks in 1992. Only heterophils and lymphocytes occurred in numbers sufficient for statistical analysis. In herring gull chicks from Saginaw Bay and the North Channel, a preliminary ANOVA provided strong evidence for a year \times site interaction effect on percent heterophils ($F_{1,48}=14.6$,

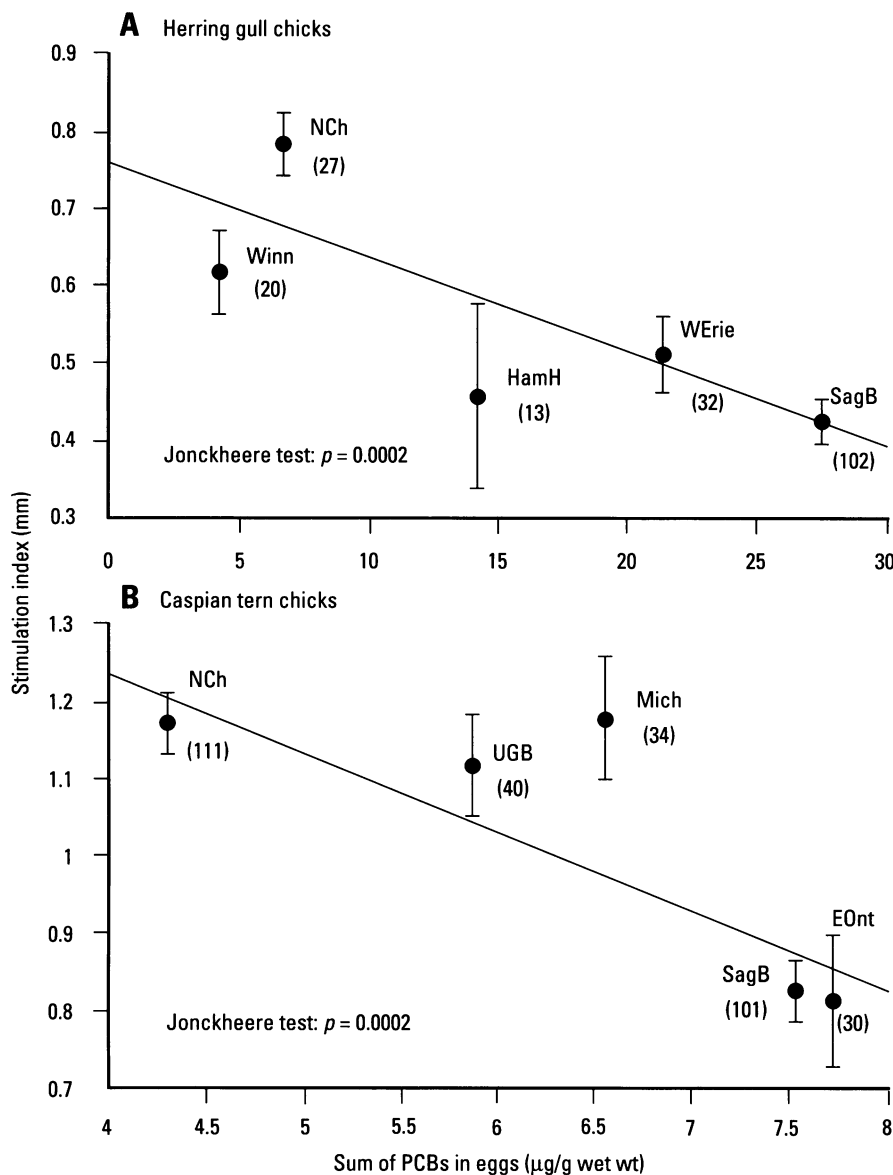


Figure 2. Relationship between T-cell-mediated immunity (PHA skin test) and PCB contamination in herring gull (A) and Caspian tern (B) chicks from the Great Lakes and Lakes Winnipeg during 1992–1994. Closed circles indicate mean response for each site. Error bars indicate one standard error of the mean. Numbers in parentheses indicate sample sizes. See Table 1 for site codes. Regression lines indicate trends.

$p = 0.0004$) and percent lymphocytes ($F_{1,48} = 13.2$, $p = 0.0007$). Considering only 1992 data, there was strong evidence that site influenced both percent heterophils ($F_{4,42} = 5.64$, $p = 0.001$) and percent lymphocytes ($F_{4,42} = 6.26$, $p < 0.0001$). There was little or no evidence that organochlorine contaminants affected the heterophil/lymphocyte ratio of herring gulls in 1992 ($p = 0.26$ for a two-way test). In Caspian tern chicks from Saginaw Bay and the North Channel, the preliminary ANOVA provided strong evidence for a

year \times site interaction effect on percent heterophils ($F_{1,53} = 48.6$, $p < 0.0001$) and on percent lymphocytes ($F_{1,53} = 29.6$, $p < 0.0001$). In 1992, there was strong evidence that site influenced percent heterophils ($F_{4,41} = 13.7$, $p < 0.0001$) and percent lymphocytes ($F_{4,41} = 7.92$, $p < 0.0001$). In 1992, there was strong evidence that the heterophil/lymphocyte ratio increased with increasing total PCBs ($p = 0.0028$ for a two-way test), C-TEQs ($p = 0.0004$ for a two-way test), and DDE ($p = 0.022$ for a two-way test).

Correlations among Functional Tests and More General Biomarkers

There was little evidence for any biologically significant relationships between T-cell-mediated and antibody-mediated immunity (Table 4). For Caspian terns in 1992 and 1992 to 1994, there was moderate evidence for a weak negative correlation of the PHA response with the total antibody titer. The PHA response showed few relationships to other biomarkers. For herring gull chicks in 1992, the PHA response was positively correlated with plasma thyroxine. This relationship was weaker during 1992 to 1993. The antibody responses were significantly correlated with WBC variables, but the nature of this relationship differed between species. During 1992 to 1993, the heterophil/lymphocyte ratio was negatively correlated with total antibody and IgG titers in herring gulls and positively correlated in Caspian terns. Plasma retinol was positively correlated with the total antibody and IgG responses in herring gull chicks in 1992. This relationship was weaker for 1992 to 1993.

Several biomarkers were correlated with measures of body size or age. In 1992, body mass and wing chord length were positively correlated with plasma thyroxine and retinol in herring gull chicks but not in Caspian terns. In herring gulls, the heterophil/lymphocyte ratio was negatively correlated with body mass and wing chord length in 1992. Weaker relationships were observed during 1992 to 1993.

Discussion

Epidemiological Evaluation

This field study provided strong epidemiological evidence for associations between perinatal exposure to organochlorines and suppression of T-cell-mediated immune function in herring gulls and Caspian terns at highly contaminated sites in the Great Lakes (Table 3; Figure 2). Suppression was most severe (30–45%) at colonies in Lake Ontario (1992) and Saginaw Bay (1992–1994) for both species and in western Lake Erie (1992) for herring gulls. Saginaw Bay, Hamilton Harbor, and the River Raisin, which enters Lake Erie near the Monroe study site, have been designated “areas of concern” by the International Joint Commission because of toxic pollution. In both species, TCDD concentrations in eggs were highest in Saginaw Bay and Lake Ontario. Support for associations between organochlorine exposure and suppression of T-cell-mediated immunity came from

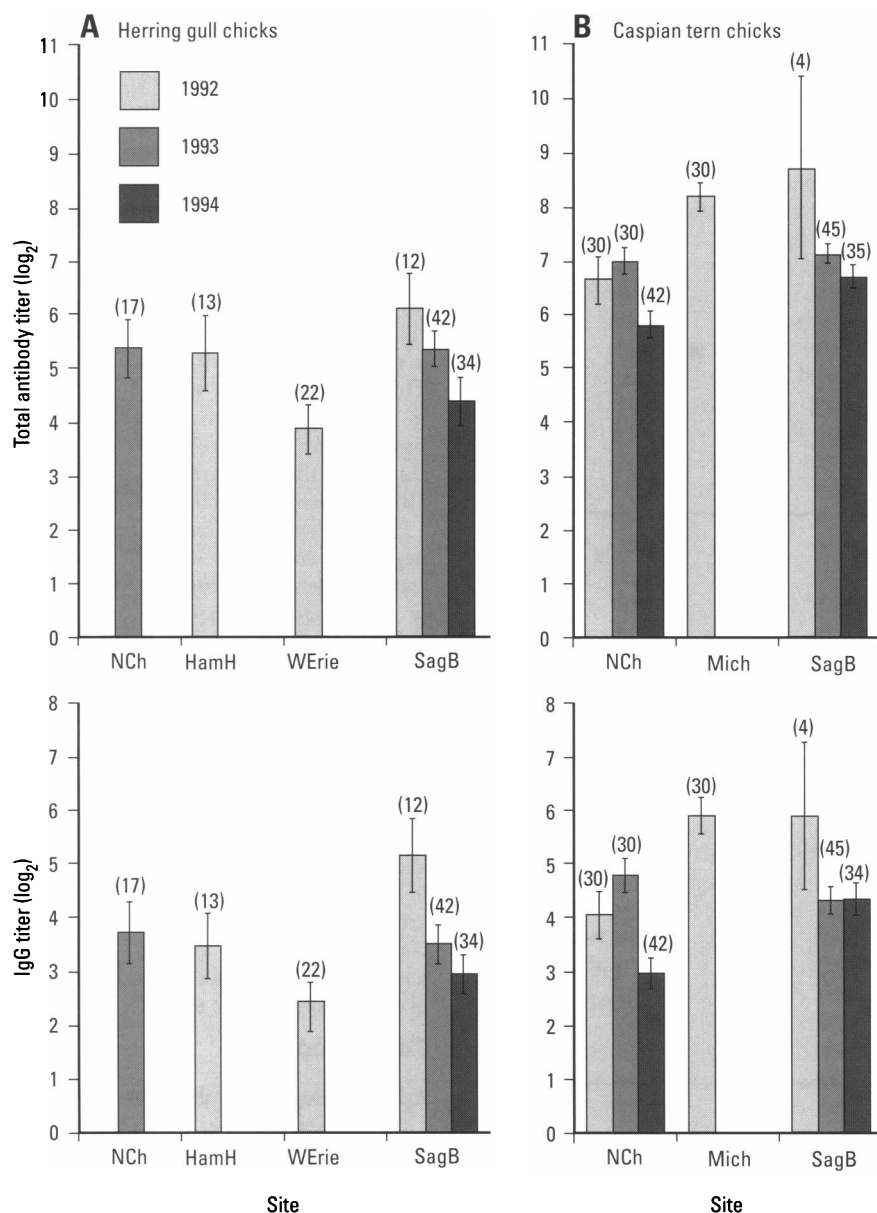


Figure 3. Mean primary antibody responses 5–7 days after SRBC immunization in herring gull (A) and Caspian tern (B) chicks from the Great Lakes during 1992–1994. Sites are arranged from left to right in the order of increasing total PCB concentrations in pooled egg homogenates. Error bars indicate one standard error of the mean. Numbers in parentheses indicate sample sizes. See Table 1 for site codes.

the following epidemiological criteria: probability, strength of association, specificity, consistency, coherence, predictive performance, and time order.

Using the criterion of probability, the Jonckheere test provided strong evidence that the PHA skin response decreased as several measures of organochlorine contamination increased ($p < 0.001$; Table 3). Beyond statistical significance, the strength of association criterion suggested that the magnitude of the suppression at the most

contaminated sites was biologically significant. Laboratory studies with birds have shown that elimination of T-lymphocyte function by irradiation or immunosuppressive drugs reduces the PHA response by 50 to 60% (75,87,88). Hence, the 30 to 45% suppression in herring gulls and Caspian terns at highly contaminated sites in the Great Lakes represents a biologically significant impact. This may approach the maximal suppression possible in these species because the weight loss in

Saginaw Bay in 1992 was not associated with further suppression. The criterion of replication strongly supported these relationships that were demonstrated *a*) in two species sharing a similar contaminated food supply (fish), *b*) at sites with similar magnitudes of contamination, and *c*) in multiple years (2–3 years) of study at some sites.

The criterion of coherence also supported an association between contaminants and suppression of T-cell-mediated immunity. Such associations are consistent with many laboratory experiments that have found severe impacts of HAHs on T-cell-mediated immunity in birds and mammals (22,26,32,33,35–47). In a parallel investigation that studied herring gull chicks at nine colonies in the Great Lakes and one colony on the Atlantic coast, thymic atrophy was associated with increasing liver EROD activity (89). Thymic mass was reduced 20 to 45% at the sites with highest EROD activity. Although thymic mass was not associated with any single organochlorine, the thymic atrophy associated with high EROD activity strongly suggests that the complex mixtures of contaminants in the Great Lakes exert toxic effects on the immune systems of young herring gulls. Our findings are consistent with the results of several marine mammal studies. Harbor seals fed HAH-contaminated herring from the Baltic Sea had reduced delayed-type hypersensitivity (T-cell-mediated) and reduced mitogen-induced proliferative T-cell responses (67,68). Mitogen-induced proliferative T-cell responses were inversely correlated with blood PCB and DDE concentrations in male bottlenose dolphins from the west coast of Florida (69).

The criterion of predictive performance supported associations between contaminants and suppression of T-cell-mediated immunity. Published laboratory studies and our own pilot field study were used successfully to predict suppression of T-cell-mediated function at colonies with high organochlorine contamination. In this pilot study during 1991, the PHA skin response was 60% lower in herring gull chicks from a highly contaminated site (Gull Island, Upper Green Bay) as compared to a reference site (Kent Island, Atlantic coast).

The specificity criterion refers both to a unique effect produced only by a single cause and to a consistent effect that always accompanies a causal factor. One difficulty in immunotoxicological studies is that immunosuppression is not specific to

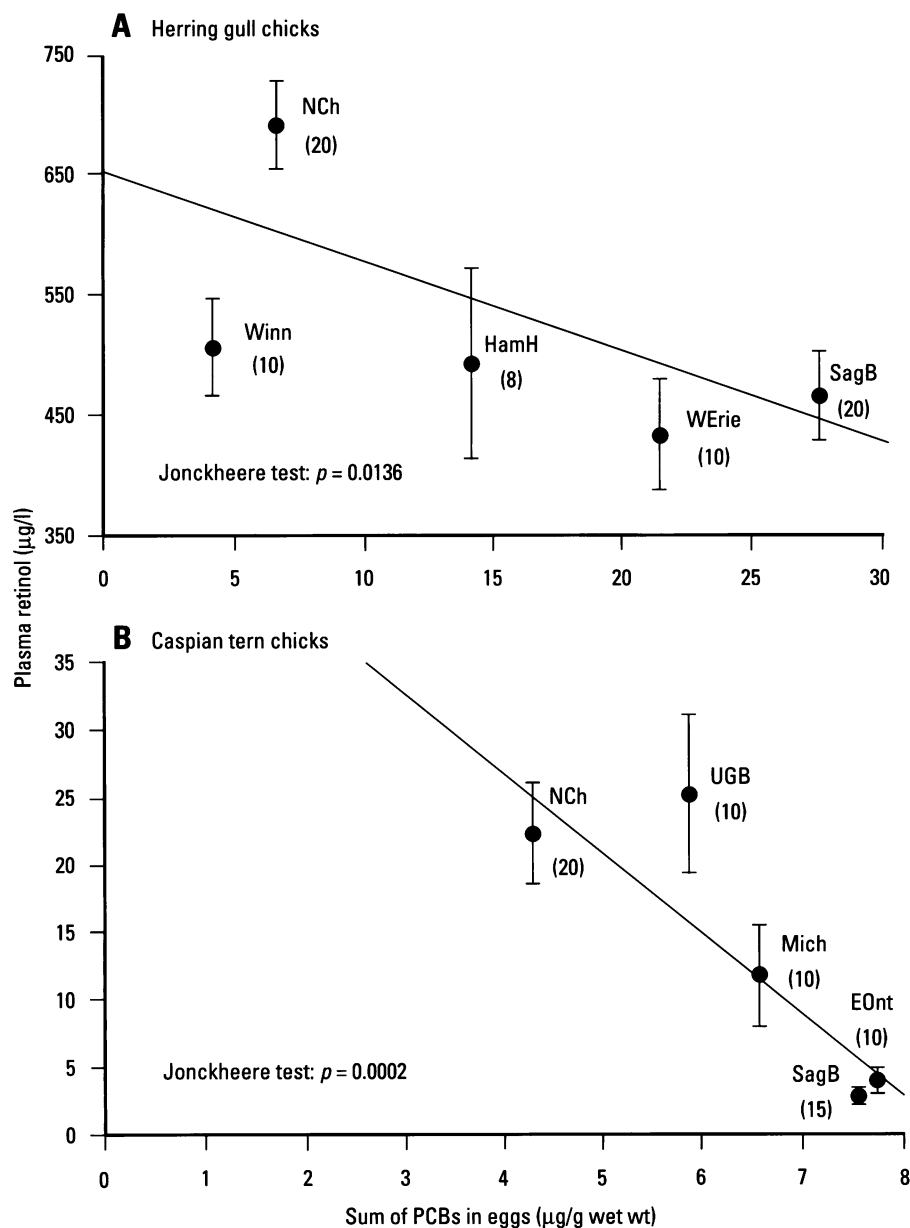


Figure 4. Relationship between plasma-retinol (vitamin A) and PCB contamination in herring gull (A) and Caspian tern (B) chicks from the Great Lakes and Lake Winnipeg during 1992–1993. Closed circles indicate mean response for each site. Error bars indicate one standard error of the mean. Numbers in parentheses indicate sample sizes. See Table 1 for site codes. Regression lines indicate trends.

pollutants; many other factors such as nutrition, stress, infections, and genetics influence immune function. However, suppression of T-cell-mediated immunity consistently occurs after developmental exposure to HAHs in laboratory animals, supporting this association in Great Lakes birds.

The time order criterion supported this association because chicks were exposed to environmental contaminants throughout development and after hatch, before T-cell function was measured. Unfortunately,

there are no data on immune function in Great Lakes fish-eating birds before the era of organochlorine pollution, so it cannot be determined whether these spatial patterns in immune function existed before this contamination. Considering that contaminant residues are approximately 80% lower today than during the early 1970s, it is likely that immunosuppression was more widespread and possibly more severe at that time.

Suppression of the PHA skin response occurred over a narrower exposure range in

Caspian terns than in herring gulls (Figure 2). The sensitivity of Caspian terns to the immunosuppressive effects of organochlorines is not surprising considering their sensitivity to other developmental effects of these pollutants (12). The lower contaminant concentrations in Caspian tern eggs are probably related to the migratory habits of terns. They migrate to southern North, Central, and South America for 6 months of the year, where they presumably eat a less-contaminated food supply when inhabiting ocean beaches. When they return to breed at highly contaminated Great Lakes sites such as Saginaw Bay, the female terns accumulate contaminants throughout the breeding season so that second clutch eggs have higher organochlorine concentrations and lower rates of hatching than first clutch eggs (12). In contrast, herring gulls are year-round residents of the Great Lakes, so they are chronically exposed to higher concentrations of organochlorines. Long-term banding studies have shown low recruitment into the breeding population of Caspian terns raised at highly contaminated colonies (19,73,74). Contaminant-associated immunosuppression provides a potential mechanism for explaining these population-level effects.

Because most organochlorines biomagnify up the food web, their concentrations tend to be co-correlated. Hence, it is difficult to determine which organochlorines were most closely associated with suppression of T-cell-mediated immunity in this study. In herring gull eggs, gradients in PCBs and DDE all occurred in the same rank order. However, PCBs occurred at much higher concentrations than DDE, and suppression of T-cell-mediated immunity following perinatal exposure is more characteristic of PCBs than DDE. In Caspian tern chicks, the PHA skin response decreased as total PCBs, C-TEQs, and DDE increased. However, the strongest association was with PCBs. PCBs had the highest concentrations of any organochlorines in Caspian tern eggs. While PCBs were most closely associated with immunosuppression in both species, effects of, and interactions with, other organochlorines cannot be ruled out.

Our epidemiological evaluation did not support the hypothesis of contaminant-associated suppression of antibody-mediated immunity (Table 3), even though the differences in total antibody and IgG titers among sites were biologically significant. Four criteria (probability, strength of association, coherence in the form of a

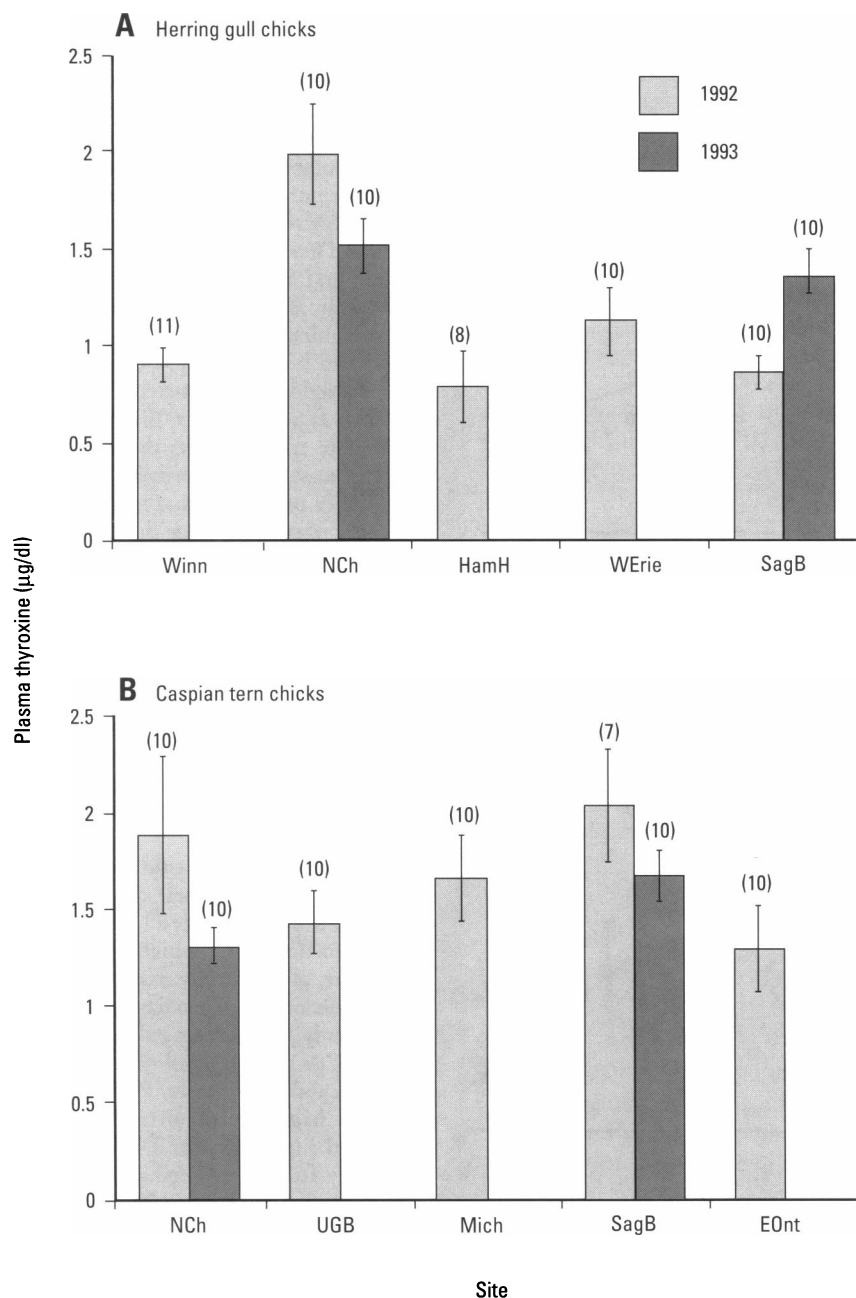


Figure 5. Mean plasma thyroxine concentrations in herring gull (A) and Caspian tern (B) chicks from the Great Lakes and Lake Winnipeg during 1992–1993. Sites are arranged from left to right in the order of increasing total PCB concentrations in pooled egg homogenates. Error bars indicate one standard error of the mean. Numbers in parentheses indicate sample sizes. See Table 1 for site codes.

dose-response relationship, and prediction from laboratory to field investigations) detracted from the hypothesis of antibody suppression. Other criteria were indeterminate. A posteriori Jonckheere tests indicated an association between organochlorine contamination and higher antibody titers in Caspian terns, suggesting contaminant-associated deregulation of antibody-mediated

immunity. Other factors such as genetics, nutrition, stress, and weather might have influenced antibody titers.

The suppression of T-cell-mediated immunity but not antibody-mediated immunity in this field study is consistent with laboratory studies on chronic and (or) perinatal exposure to HAHs. PCBs most consistently suppress antibody-

mediated immunity at high acute doses rather than at the chronic developmental exposures observed in this field study. In harbor seals fed HAH-contaminated herring, antibody responses to ovalbumin were reduced (68), but mitogen-induced proliferation of B cells was not affected (67). B-cell proliferation showed no associations with organochlorines in male bottlenose dolphins from the west coast of Florida (69).

Although the anti-SRBC antibody response requires helper T lymphocytes, the suppression of T-cell-mediated immunity but not antibody-mediated immunity in this field study is consistent with current immunological theory. The contaminant-associated suppression of the PHA skin response may reflect suppression of a subset of helper T lymphocytes that boost inflammatory responses but not antibody-mediated responses. In mice, T_H1 cells promote inflammatory cytotoxic responses while T_H2 cells promote antibody responses. Suppression of the PHA skin response in herring gulls and Caspian terns suggests suppression of T_H1 -like cells that would not participate in antibody responses. In laboratory animals, HAHs have been shown to suppress such inflammatory (delayed-type hypersensitivity) and cytotoxic responses (24,32,33,42,45). In many cases, these cell-mediated responses are suppressed without any effects on production of antibodies that depend on helper T-cell (T_H1) activity (33,43,45).

Evaluation of Immunological and Biochemical Biomarkers

The PHA skin test was an extremely effective and sensitive biomarker for assessing T-cell function in wild birds. Low-within-site variation allowed statistical differentiation among sites, especially when sample sizes were greater than 20 birds per site. At sites where the test was replicated for 2 or 3 years, the response was consistent. For both species in Saginaw Bay, immunosuppression was similar during the year of severe body mass loss and mortality (1992) compared to the following 2 years of better growth and fledging success, suggesting that T-cell-mediated immune function is a more sensitive end point. In northern bobwhites (*Colinus virginianus*), the PHA skin test was more sensitive to the immunosuppressive effects of a low protein diet as compared to *in vitro* proliferation of T lymphocytes in response to PHA (90). The *in vitro* T-lymphocyte proliferation assay measures only very early events in the T-cell

Table 4. Pearson's correlation analysis exploring relationships among biomarkers in herring gull and Caspian tern chicks from the Great Lakes and Lake Winnipeg during 1992 to 1994.

Relationship	Species	Year	Variables		<i>r</i>	<i>n</i>	<i>p</i>
Among measures of immune function	Caspian tern	1992	PHA	Antibody	-0.31	63	0.015
		1992–1994			-0.16	214	0.017
Among immune function and other biomarkers	Herring gull	1992	PHA	Thyroxine	0.32	47	0.026
		1992–1993			0.21	67	0.085
	Herring gull	1992–1993	Antibody	H/L ratio ^a	-0.50	37	0.002
				IgG	-0.56	37	<0.001
	Caspian tern	1992–1993	Antibody	H/L ratio	0.41	58	<0.001
				IgG	0.35	58	0.007
	Herring gull	1992	Antibody	Retinol	0.68	15	0.006
		1992–1993			0.31	31	0.09
		1992	IgG	Retinol	0.55	15	0.033
		1992–1993			0.22	31	0.24
Among body size and biomarkers	Herring gull	1992	Thyroxine	Body mass	0.36	48	0.013
		1992–1993			0.23	69	0.051
		1992	Thyroxine	Wing chord	0.45	48	<0.001
		1992–1993			0.37	69	0.002
		1992	Retinol	Body mass	0.36	48	0.013
		1992–1993			0.26	68	0.03
		1992	Retinol	Wing chord	0.45	48	<0.001
		1992–1993			0.28	68	0.019
		1992	H/L ratio	Body mass	-0.43	47	0.003
		1992–1993			-0.32	80	0.004
		1992	H/L ratio	Wing chord	-0.39	47	0.007
		1992–1993			-0.25	80	0.027

^aHeterophil/lymphocyte ratio.

response that are involved with cell division. In contrast, the *in vivo* skin test incorporates numerous events in the T-cell response, including cell proliferation, differentiation, and cytokine production (90,91).

The SRBC hemagglutination test was a good biomarker for measuring antibody-mediated immune function in wild fish-eating birds, allowing statistical differentiation among sites. The IgG titers usually gave smaller *p*-values in ANOVA analyses as compared to the total antibody titers, suggesting that IgG is a more sensitive biomarker. A disadvantage of this assay is the need to make two visits to each colony 6 days (or 5–7) apart to collect blood at the peak of the antibody response. Poor weather and other logistical problems can make it difficult to return to a colony during this narrow time window, especially if immunological tests are being conducted simultaneously at several sites.

Where the hemagglutination test was replicated for 3 years, the response often changed over time. Time trends in antibody titers were confounded by a change in the individual sheep that served as a source of SRBCs. The sheep used during 1992 to 1993 died before the 1994 field season, so

a sheep of similar age from the same flock was substituted. In Saginaw Bay herring gull chicks, a nonsignificant decrease in total antibody titers occurred over 3 years. The IgG response decreased significantly from 1992 to 1993 but not from 1993 to 1994. In Caspian tern chicks from Saginaw Bay and the North Channel, the total antibody response dropped from 1992 to 1993 and again from 1993 to 1994. The IgG response showed a significant year \times site interaction. North Channel IgG titers dropped significantly from 1992 to 1994, although the 1993 titers were not significantly different than the early or later years. Saginaw Bay IgG titers dropped significantly from 1992 to 1993, but not from 1993 to 1994. In both herring gull and Caspian tern chicks from Saginaw Bay, IgG titers dropped between 1992 and 1993 while the same source of SRBCs was used. This drop in IgG coincided with a doubling in total PCBs and DDE in herring gull eggs from 1992 to 1993 (DV Weseloh, personal communication).

Few general biomarkers were correlated with measures of T-cell-mediated or antibody-mediated immune function, suggesting that they were not mechanistically

responsible for the immunosuppression observed (Table 4). Although retinol and thyroxine often influence immune function in laboratory studies, there was little evidence that these variables were good surrogate biomarkers for immune function in this field study. Plasma retinol was strongly correlated with antibody responses in herring gull chicks in 1992 but less so when 1993 data were added. Retinol did not appear to influence immune function in Caspian tern chicks, although retinol concentrations were one to two orders of magnitude lower in terns than in gulls. Apparently, physiological regulation and (or) dietary intake of vitamin A differs greatly in these two species. Nonetheless, in both species plasma retinol decreased as PCB contamination increased, exhibiting a similar association with contamination as the PHA skin test (Figure 4). However, in herring gull chicks this association was strongly influenced by the North Channel site, which had much higher retinol values than Lake Winnipeg, the other reference colony. Plasma thyroxine showed a strong relationship to the PHA skin response in herring gull chicks in 1992, although this relationship was weaker when 1993 data

were added. There was no evidence for such a relationship in terns.

Although the tests of immune function were not confounded by growth, several other biomarkers were related to body size and (or) to length of exposure to pollutants, which increases with growth. Larger herring gull chicks tended to have higher concentrations of plasma thyroxine and retinol and more lymphocytes than heterophils. These relationships were evident even in chicks that fit body mass and wing chord criteria for approximately 21 days of age.

The heterophil to lymphocyte ratio integrates differential counts of the most abundant WBCs in birds. With respect to disease resistance, this ratio quantifies the balance between the nonspecific, fast-acting defenses of heterophils and the antigen-specific, slower acting defenses of lymphocytes. Heterophil to lymphocyte ratios are increased by stress (92) and may be

influenced by other factors. The two species displayed different relationships between differential WBC counts and antibody responses. In herring gulls, the antibody responses increased as number of lymphocytes, some of which produce antibodies, increased relative to the number of heterophils. Conversely, in Caspian terns the antibody responses increased as the relative number of heterophils to lymphocytes increased. Total lymphocytes counts might clarify the antibody relationships to differential counts, but total WBCs can be difficult to count under field conditions.

Conclusions

This study demonstrated contaminant-associated suppression of T-cell-mediated immunity in prefledgling chicks of two species of fish-eating birds from the Great Lakes. Suppression was most severe at colonies in Lake Ontario (1992) and

Saginaw Bay (1992–1994) for both species and in western Lake Erie (1992) for herring gulls. The identity of the particular organochlorine(s) responsible for such suppression could not be determined since exposure to different organochlorines was correlated due to similarities in environmental chemistry and metabolism. However, PCBs were the most closely associated with immunosuppression. Additional research is needed to determine the relationship between suppression of functional assays and increased susceptibility to infectious diseases. Such research will be important for determining the consequences of T-cell-mediated immunosuppression on individual survival and population dynamics. Alterations in variables such as retinol and WBC counts suggest biologically significant differences in physiology and health among various Great Lakes colonies.

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